to six times as much, proportionally to the weight of the animal, was swallowed.

The Society then adjourned over the Easter Recess, to Thursday, May 2.

May 2, 1878.

Sir JOSEPH HOOKER, K.C.S.I., President in the Chair.

The Presents received were laid on the table, and thanks ordered for them.

In pursuance of the Statutes, the names of the Candidates recommended for election into the Society were read from the Chair as follows:—

John Gilbert Baker, F.L.S.
Francis Maitland Balfour.
Rev. Thomas George Bonney, M.A.
Prof. James Henry Cotterill, M.A.
Sir Walter Elliot, K.C.S.I.
Rev. Canon W. Greenwell, M.A.
Thomas Hawksley, C.E.
John Hopkinson, M.A., D.Sc.
John Hughlings Jackson, M.D.
Lord Lindsay, P.R.A.S.
Samuel Roberts, M.A.
Edward A Schäfer, M.R.C.S.
Herman Sprengel, Ph.D.
George James Symons.
Charles S. Tomes, M.A.

The following Papers were read:—

I. "On the Life-History of a Minute Septic Organism: with an account of Experiments made to determine its Thermal Death Point." By Rev. W. H. DALLINGER. Communicated by Professor HUXLEY, Sec. R.S. Received March 26, 1878.

[PLATES 8, 9.]

Nearly four years since, in examining an infusion of animal matter, which had, unfortunately, been diluted with water, and had vegetable substances placed in it, I observed a minute and intensely active organism, which, on closer and more careful examination, I found to be of a form entirely new to me. I therefore determined to endeavour to discover its life-history; but the diluted infusion was unsuited to it, and in the course of five days no living form remained. During this time I had been able to determine very little that was consecutive, but had seen enough to lead me to desire to find it again; and for the two following years I steadily examined all the infusions within my reach, as well as all probable places besides, but in vain. And it was

not until the latter part of the summer of 1876 that I found it again. It appeared then, in a maceration, in which the body of a vole was decomposing. The maceration was only three weeks old, and this organism had evidently only just arisen; it increased daily in vigour and numbers, and in three weeks I was at liberty to study it continuously.

For this purpose I employed the "continuous stage," jointly devised for preceding researches,* by means of which a drop of the infusion could be kept under examination, without evaporation, for an indefinite time, and with the most powerful lenses.†

My method was to follow out, as far as possible, the morphological details separately; and then to steadily follow one form from its earliest to its most mature condition; thus discovering how the different states were related, and making out an unbroken life-history.

Most of the more difficult and delicate work was done with an entirely new lens, made specially for me by Messrs. Powell and Lealand; and which is nominally a $\frac{1}{35}$th inch lens. It was made specially for this class of investigation, for which it is admirably adapted. Its "working distance" is sufficient; its "penetration," for such a power, is extremely great, its angle being moderate; and its "definition" is as sharp and clear, when properly used, as that of the finest $\frac{1}{4}$th or $\frac{1}{8}$th. I also had the advantage of the use of the four new lenses made on a "new formula" by the same makers, viz., a $\frac{1}{8}$th of low angle and great penetrating power; and a $\frac{1}{8}$th, $\frac{1}{12}$th, and $\frac{1}{16}$th of high angles. I also used the $\frac{1}{25}$th and $\frac{1}{50}$th lenses.

One of the difficulties attending the study of this organism was its extreme rapidity and caprice of movement. Its normal form is depicted at fig. 1, Plate 8. It is there magnified 3,000 diameters. Its sarcode is clear, and to all the lenses employed structureless. It is usually found to have minute vacuoles scattered through it. The form of the body is distinctive. Its greater part is a long oval slightly constricted a little above the middle. But from the front, or shorter portion, a kind of neck (*a*, fig. 1) protrudes, from which proceeds the front flagellum, which is extremely fine, and from one and a-half times to twice as long as the body. Below this and at the sides or "shoulders" of the organism, two other long and fine flagella arise, proceeding backwards, as seen at *b*, *c*, *ibid.* In addition to this, there is always a nucleus-like body slightly to one side of the lower part of the organism, as seen at *d*; and with the higher lenses and delicate manipulation there was frequently seen, within this, an ex-

* *Vide* "Further Researches into the Life-History of the Monads." Monthly Micro. Journal, vol. xi, pp. 97–99.

† Linen instead of bibulous paper is now used as the agent for conveying a constant supply of evaporating moisture to the chamber in which the organisms are examined in this piece of apparatus.

tremely minute globule, also indicated at *d*. But as this was not always present, even in the normal state of the organism, it was probably not important.

The extreme length of the body was the $\frac{1}{4000}$th of an inch. It rarely exceeded this and was often less.

It swam, as I have said, with great rapidity; and all its movements were most graceful, varied, and controlled. Its usual mode of motion is in a direct line, or in curves; and with its trailing flagella behind and its active anterior one, it is a very remarkable object. But the suddenness with which it can arrest its most rapid movement, or change it in any direction up, down, or directly reversed, is still more remarkable. In this the flagella at the sides are brought into active operation; sometimes both being spread out like long arms; at other times one being stretched out and in vigorous action, while the other is closely pressed to the other side, and so forth, giving this organism a control over its movements which is of extreme interest. Indeed the apparent volitional mastery which this seemingly structureless speck exercises over these equally structureless filaments, for the determination of its course, cannot be seen and studied without wonder.

But besides this free-swimming movement, it was capable of the most vigorous motion in an "anchored" state. Most of the larger septic organisms belong to, or are associated with, certain conditions of the decomposing matter; and disappear before the advent of other forms, when that condition is past. This one was associated with the general breaking up of the decomposing animal matter; and the movement which I am about to describe apparently contributed to this. It was a powerful springing motion. The trailing flagella became attached to the floor as at *a*, *b*, fig. 2, by what means could never be discovered; but the attachment was very secure and could be discontinued at any moment. Directly the anchorage is made, the two anchored flagella with the utmost rapidity coil in a spiral, as at *c*, fig. 2, bringing the body of the organism nearly to a level with the floor. With equal suddenness it darts up in the line indicated by the arrow *d*, and thus having reached the limits of its flagella it moves down, with equal rapidity, in an arc of a circle of which the flagella are the radius; indicated by the arrow *e*. In this way the whole body is brought down on the point *f*. But the matter to be noticed is, that this is never done except upon a fragment of the decomposing tissues in the infusion. And if a small fragment be taken out at any time and examined, it would soon be encircled by thousands of these forms incessantly darting up and down upon it in the way described; and careful observation showed that they, by this means, rapidly changed the form and diminished the size of the little particles so attacked. No morphological changes are at all apparent in those that

are in this condition, but they are continually freeing themselves and swimming away and others are constantly coming.

The changes now to be described will be understood to be given as the result of long-continued and repeated effort. Failure is very much more frequent than success in working out the preliminary details, but pre-eminently so in steadily following to the end the changes undergone by a minute organism.* I merely record the final results, which have issued both from the study in detail of phases in the life-cycle of the organism, such, for example, as the minutiæ of the method of fission and fusion—and also from an unbroken observation of its comparatively short life-cycle, which was three times repeated.

Following then, steadily, a normal form, which has just freed itself from the springing condition, as fig. 1, the first real indication of change, though by no means the first to be *discovered*, is a splitting of the anterior flagellum, as seen at *a* and *b*, fig. 3, and the moving of the nucleus to the centre, as seen at *c*. With a power of from three to four thousand diameters, there may also be seen a delicate line under the base of the flagellum, as shown at *d*. In the course of from thirty to sixty seconds this has widely opened and the base of the flagellum has divided as seen at *a*, fig. 4, while at the same time the nucleus shows an incision in the direction of its length, as seen at *b* in the same figure; and a similar incision has commenced at the posterior end of the body, as shown at *c*. In a very few seconds more, this slight incision (*c*, fig. 4) is the origin of a wide opening, as seen at *a*, fig. 5, above which it will be seen that the nucleus has almost divided and a pale line runs through the body-substance from the upper to the lower opening. The posterior opening widens much more rapidly than the anterior one, as fig. 6 shows, depicting a condition ensuing in from one minute to four minutes after that shown in fig. 5. And at this stage the nucleus has split into two and the divided parts occupy distinct positions, as shown at *a*, *b*. The upper or anterior split now widens as well as the posterior one, as shown in fig. 7, leaving the two parts merely united by a neck of sarcode.

* In fact hundreds are followed which from one or more of many causes do not complete the cycle of their lives. It may be arrested—and frequently is—by death in the earlier stages, in the middle, or still worse towards the end. In the same way there may be failure by the individual form being lost amidst a crowd of others, or by slowly working its way to the little ring of liquid at the edge of the "cover," and then going out into it, and so making further study impossible. Or the apparatus may be at a critical moment a source of trouble; the delicate balance between the moisture carried over by capillarity, into the chamber containing the fluid with its organisms, and the evaporation taking place, may by some means, such as a sudden change of temperature, be broken, and nullify hours of patient and, but for that, successful work. In every such case there is but one method open—it is to begin again *de novo* upon another form.

Great vigour of movement now ensues, but of an irregular kind; and instead of being forward, is from side to side; when suddenly the distance between the two parts widens, as in fig. 8, a stretched fibre of sarcode alone uniting them. Movement is still apparently concerted, and is *from* each other, the fibre of sarcode becoming speedily thinner until it has stretched to double the length of an ordinary trailing flagellum; this is shown in fig. 9; and in a few seconds more, by a vigorous movement of each body in opposite directions, as the arrows indicate, the fine filament of sarcode snaps in the middle, and two perfect forms are thus set free by fission.

The process is carried on with great vigour, and is *apparently* the only metamorphosis to which the organism is subject. In nine separate cases a single form was carefully followed, that is to say, a vigorous form in each case was watched from its first act of fission; one part of the divided organism being constantly followed. The whole process of division, from its visible beginning to its close, took place in from four to seven minutes; and was recommenced during the first hour in periods not exceeding three minutes. For the next two hours the intervals would be from seven to ten minutes, and after this the intervals became indefinite, not being less than twenty nor more than forty minutes.

Following the first separated segments, as represented by one part of its divisions, in all its subsequent separations, as begun and carried on by nine separate organisms in which fission happened for the first time, the terminus of the process of fission in the last of the segment-forms in all the cases, was *death* in six of the nine instances, and entire metamorphosis in the remaining three. In the cases in which death ensued the process of fission was continued for seven hours in one case; six hours in three cases; and five-and-a-half hours in the remaining two. In the cases in which metamorphosis took place, three hours in one case, and four hours in the remaining two, terminated the period of fission. There was then inaction in this last segment-form, and the appearance indeed of the loss of vitality; but the first indication of a difference between this and the more frequent cases of vital collapse, was the rapid "clubbing," or gathering into knots of the trailing flagella, followed by a rapid enlargement of the nucleus, and a glittering, rapidly amoeboid condition of the entire body. This condition is shown in fig. 10. The changes are now very rapid: not more than seventy seconds elapse before the trailing flagella are wholly fused with the body substance, and while the head-and-neck-like protuberance pointed out in fig. 1, is preserved, the body, having lost the lateral or trailing flagella, becomes oval; with an immensely developed nucleus, as shown in fig. 11. It swims now with great ease, but always merely in a straight line, and always takes a sudden dart backwards before changing the direction of its motion. It may swim in this way for from a quarter to half an hour; but during this time a band of gra-

nules is formed, at first faintly, and afterwards very distinctly, which is shown at *a*, fig. 11. In this condition it swims directly into the midst of a group of forms in the springing state shown in fig. 2, and the utmost care is needed to keep it in view; but as a rule not many seconds ensue before it has firmly attached itself to one of the springing forms, which at once unanchors itself, and both together swim freely and vigorously about, as shown in fig. 12. They now swim in concert for a very variable period, but generally from thirty-five to forty-five minutes, when their movements become sluggish; the trailing flagella of the lower form become inert and fall upon, and become fused with, the mass of sarcode; meanwhile the bodies have rapidly been uniting, and the two nuclei become fused together, as seen in fig. 13, while the anterior flagella become, in their extremely sluggish movements, at last, entangled with each other, and also melt together, as shown at *b*, and all movement ceases. In the course of ten minutes, from this condition, all trace of the separate bodies is lost, a more or less regular oval form is taken, as shown in fig. 14; at the same time a slow amoeboid disturbance of the sarcode is visible, which diminishes until perfect fusion results, which occurs in the course of twenty minutes, all trace of the nuclei having vanished; and the oval gradually elongates until it reaches the tight, glossy, still condition shown in fig. 15.

This, as previous experience had led me to expect, proved to be a cyst charged with spores. On the first occasion, I anticipated a long continuous watch; but at the end of three hours there was a sudden falling in the middle of the spindle-shaped sac, and a general alteration of form, which led me to earnest effort to discover some discharge. And that it was taking place there could be no doubt, for there was a rapid diminution in the size of the sac; and there were apparent fractures in the delicate investing membrane; but nothing more than cloudiness at the ends could then be made out. I was using the $\frac{1}{16}$th new formula lens (dry), and exhausted all expedients, in vain, to make out the real cause of the change; and as it is hopeless to attempt to change a lens successfully under such circumstances, there was nothing to be done but await another opportunity; using another lens. This $\frac{1}{16}$th had proved of great value throughout in the detection of minute structure and change of form; but the kind of illumination which best reveals extremely minute semi-opaque bodies, is the perfect central illumination which I have endeavoured to describe in the "Monthly Microscopical Journal," vol. xv, page 165. That is to say, the illumination of the whole field through a circular aperture in the diaphragm from the $\frac{1}{80}$th to the $\frac{1}{100}$th of an inch in diameter, through the centre of which aperture the optical axis of the sub-stage condenser, if continued, would pass. But for this special method the new $\frac{1}{16}$th is not so efficient in my hands as the $\frac{1}{25}$th, $\frac{1}{35}$th, or $\frac{1}{50}$th.

Having, therefore, ultimately secured another sac, I determined to use the $\frac{1}{35}$th, as being, as I believed, the most suitable lens for the purpose. In the still condition of the sac, ample time was given for the most delicate adjustment of the light and of the lens, and the watching was begun under the best conditions.

Not the slightest movement, either within or without, is visible while the cyst is in this condition; and the time over which this inactivity extended was never less than three hours, nor more than five hours, in the eight separate instances which I studied. In the second instance the length of time which elapsed was four hours. At the end of that time, without reason discoverable at the moment, there was as before a falling in of the centre of the cyst, and an irregularity in its shape, with a visible and rapid diminution in size. At first nothing but a cloudiness at the two ends could be discovered; but, by a little delicate adjustment of light and lens there became distinctly visible an outflow, from the two ends and the middle of the cyst, of the most exquisitely minute particles which I have ever seen; and as the quantity flowing out diminished, their segregation became more complete, and their clearness and independence more manifest.

The nature of the "continuous stage"—which keeps the drop of fluid under examination from evaporating—is such as to compel the use of the microscope in an upright condition, in order that the stage of the instrument may be perfectly horizontal. On this account the ordinary "camera lucida" is of no avail, or only of use by special and difficult arrangements; but a beautiful little instrument, made by M. Nachet, of Paris, specially meets this emergency, and enables us to make camera lucida drawings with great ease and accuracy. I had, therefore, by anticipation, arranged this apparatus: and when the outflow had continued for about five minutes, made a drawing of the cyst, of which fig. 16, Plate 9, is an accurate copy. As, of course, the minute particles were in rapid movement of outflow, they were put into the drawing, not with camera lucida, but subsequently; and they accurately represent what was seen at the time. The emission continued for about forty minutes, becoming more and more feeble, until at last there was no more movement. At this stage there were many of these minute particles well segregated round the ends of what remained of the cyst; and then I was able, not only to make a drawing of the delicate white film of the cyst, but to indicate by fine dots the relative appearance of the cyst and the still particles. This drawing is reproduced at fig. 17.

The entire details of the subsequent history of these minute bodies were of course only made out by several successive and continuous observations on the emissions of different cysts. But, in each case, the lens was fixed upon some of the minute bodies that remained near the exhausted cyst, and then the observation was unbroken

and continuous to the end. When first seen, these exquisitely minute specks are strongly opaque; but as they are attentively watched with a magnification of 5,000 diameters, they, in the course of from twenty to forty minutes, become quite clear, and would be much more difficult to find in this condition than when first poured out. But, following upon this, there is a distinct elongation, which is indicated at fig. 18, and this proceeds so that, at the end of an hour and a half, they were in the shape and relative size shown at fig. 19.

On the first and second occasion on which the observations were made, very little was made out, except the succession of the metamorphoses; but on subsequent occasions, when a careful scrutiny for the discovery of delicate changes was made, the clear, sharp, defining power of the new $\frac{1}{16}$th objective was of great service; by its means I was enabled to discover the first indication of the origin of the anterior and lateral flagella in the developing germ. At the end of an hour and a half after emission from the sac, the long oval form which the germ had taken (indicated at fig. 19) was always associated with a sharpening at one end. This became rapidly sharper and longer, as seen in fig. 20, at 4 and 6. This always developed into the anterior flagellum. But at about the same time—generally about two hours after emission from the cyst—there were pushed out two delicate points of sarcode laterally, and at right angles to the beak in front; 1, 2, 3, 5, fig. 19, are copies of camera drawings of this condition in the minute germinating body; and the extremely delicate lateral points were found by close watching to develop into the lateral flagella of the perfect form. The growth was now much more rapid; fig. 21 represents the changes that had taken place in forty minutes after the condition depicted in fig. 20. And now slight movements began in some of the better developed forms, such as *a*, fig. 21. They were not changes of place, but movements of the lateral and anterior flagella, and occasionally a change of the direction in which the bodies lay. In the course of another hour the neck-like protrusion at the base of the anterior flagellum generally appeared as shown in *a*, *b*, fig. 22, and very soon—in every instance less than a quarter of an hour after—short movements of translation begin, which resulted in ten minutes more, in graceful swimming. Fig. 23 is a copy of a drawing made of one of these forms that had reached the condition the drawing depicts in a little over four hours from the time of emission from the sac. It was then the $\frac{1}{8000}$th of an inch in long diameter, just one-half the size of the adult; but it was perfect in form, even to the possession of a nucleus, and swam with freedom and grace. Its increase of size from this time was even more rapid, for in the course of another hour it had passed through the size and condition seen in fig. 24, to the normal parental size depicted in fig. 25. And if, after it had reached this state, it were still persistently followed, it might be in ten or

fifteen minutes, but certainly within an hour, the first signs of fission would show themselves, as indicated in *a*, *b*, *c*, *d*, fig. 25 ; and from that time fission, in successively separated individuals, continued in active progress for hours, as before described.

Thus the life-cycle was completely made out.

It may be noticed :—

1. That the method of fission is both delicate and complex, being not merely a separation into two parts of a particle of living protoplasm, but a division of distinct organs, such as nucleus and flagellum.

2. That the transformation of the individual form, at the terminus of a series of fissions, into an apparently new organism, prior to genetic blending (as shown in figs. 10, 11, Plate 8), is remarkable. And

3. That the life-cycle of this form is rigid, and marked by no caprice. It passes regularly through the same metamorphoses. If seen in any given state, it could be positively affirmed through what stages it had passed, and through what remaining stages it must subsequently pass, to reach the adult condition.

It now remained to determine whether the spore, or germinal particles, emitted by the sac, possessed any capacity to resist the action of heat not possessed by the fully developed form.

It was first carefully determined, by a method to be presently described, that the adult organism never survived after exposure for five minutes to a temperature of 142° F.

For determining the amount of heat resistance possessed by this spore, two methods were employed :—

1. The ordinary three by one inch slips of glass, used for microscopical purposes, were cut into three pieces, each of which was thus an inch square. These could be put into and worked with the "continuous stage." The septic fluid containing the organism was placed upon it, and covered in the usual way with the thinnest covering glass. It was then watched with suitable lenses until, either in that or in some successive drop of the fluid, a cyst was seen to pour out its spores, as in fig. 16, Plate 9. It was then at once taken and placed in a small brass chamber, into the middle of which the bulb of a delicate thermometer was fixed, the tube fitting air-tight into the cover of the chamber, and the expansion of the mercury being read off outside.

The small square of glass containing the fluid and spores was placed, as it came from the stage of the microscope, in the middle of the chamber. The chamber was then closed, and heat so applied as to slowly raise the temperature of the air within. In half an hour it had reached the temperature I desired, which was 210° F.; and the chamber was kept accurately at this point for ten minutes.

The square of glass was then taken out, cooled, and examined, and, of course, to the lens presented a dry amorphous aspect.

Previously some of the fine capillary tubes known as "vaccine tubes"

had been filled with water, to which a little filtered fowl broth was added as nutriment. These tubes were then hermetically sealed, and heated up to 310° F. in a digester. These were kept in absolute alcohol, so that the outsides of the tubes might not contract dust, and so forth.

The contents of these tubes were now used to remoisten the dried film beneath the cover-glass. The ends of the tubes were broken off, and by capillarity the fluid was drawn beneath the cover, at the edge of which the now open end of one or more of the tubes was applied. In this way a plentiful supply of moisture was given.

It was now placed again in the "continuous stage" and examined. At the end of four to five hours the fluid film looked almost everywhere clear, and as it appeared before heating; but I was not able by any method I employed to discover the minute spores, nor could I find in this one the earlier stages of development; but at the end of seven hours, while "searching" with a low angled $\frac{1}{12}$th inch objective, I saw three forms in about the state of development shown in fig. 23, Plate 9; and in the course of another hour a great increase in their number had taken place, and in three separate instances they were followed from the time they were first seen into full development and the commencement of fission.

When more cysts were found this entire process was again repeated twice at the same temperature, and at the expiration of ten and eleven hours respectively adult forms in full activity were found; and in the latter of these two instances I discovered the organism when it was in the still condition of development, represented in figs. 20 and 22, Plate 9. It was thus manifest that by this method of heating a temperature of 210° F. could be completely resisted.

Following the above process in every particular, I next, as they could be found, subjected the spores to 260° F. I did this on three glass squares (as before) in succession. But in no one of these did the organism reappear, although examination was repeatedly and carefully made for five days.

It thus appeared that a temperature of 260° F. applied in this way was fatal to the form, for on taking a needle-point of the septic fluid containing the organism in abundance, and, touching the edge of the fluid with it, I found that in the course of four or five hours there were hundreds swimming in full vigour, showing that the fluid was capable of sustaining the organism, if it were in a vital condition in the fluid.

I next experimented at 10° F. lower, viz., 250° F. I did this on the contents of two separate cysts. In the one case, after ten hours, there was a re-appearance of the living organism, but only two or three adults could be then found; and the increase that afterwards ensued was dependent upon fission, for no small forms could be seen. In the other case, there was rather a larger number of adults at the end of

between nine and ten hours, but their number was far fewer than in those instances where a lower temperature had been employed.

I followed this with four more experiments, separately and successively made. Two of them were at a temperature of 248°, and two at 252° F. In both of the former, at the end of nine or ten hours, the complete organism in full vigour could be seen; and, in one of the cases, it was discovered in the still condition shown at fig. 20, Plate 9, and watched until the organisms had attained the condition indicated in fig. 24, Plate 3.

But in the two latter instances (heated up to 252°) the living form did not re-appear during the six days following, although repeatedly looked for.

I concluded, therefore, that the temperature of 250° F. was the limit of endurance which the spore of this form could bear by this method of heating.

Now, it must be noticed that in testing the death-point of the adult, as I shall presently show, a fluid heat was employed. But the spore can hardly be said hitherto to have been heated in the same conditions. Doubtless, in the heating chamber the higher temperatures are endured after evaporation, and consequently the heat is "dry." But after years of thought on the matter, no other way has suggested itself to me as possible, for the attaining of the result, by other means. What is required is, that we should know beforehand, that in the drop of fluid exposed to a given heat, the spores of a given organism *in a freshly emitted condition*, and, therefore, before development had begun, were present. And, moreover, that it should be so placed that after heating it should be in such conditions as would admit of the use of the highest power lenses to discover if the spores, known to have been deposited there, would, after subjection to the thermal conditions given, develop as they had been demonstrated to do before heating.

Now, there are two ways of doing this. The one is approximate. It is by long and intimate acquaintance with the history of the organism, and a careful and close study of the condition of the fluid—by which means it is possible to come very near to the highest probability—that the spores in the required condition are there. But in this case negative results must be extremely perplexing. The other way is to see the spores actually emitted in the drop of fluid that is to be heated. This is the only certain method; it is most exhausting and laborious, but its results are certain, and therefore I have employed it in this case. But to adopt the supposition which Dr. Bastian has recently done,* that to heat a given monad spore in a given condition

* On the Conditions Favouring Fermentation, &c., "Linnean Soc. Jour.," vol. xiv. This paper contains some curious inaccuracies of statement as to facts—evidently the results of misapprehension on Dr. Bastian's part—concerning the results of thermal experiments made by Dr. Drysdale and myself on the Monads. These occur

in a fluid is "perfectly easy," is a serious mistake. He thinks that we have nothing to do but to "put one or two drops of the fluid into a small tube . . . . hermetically seal it, and then heat it for ten minutes or more to different degrees before subjecting the fluid to a prolonged microscopical examination."

Certainly this is a "perfectly easy" method; but it must fail entirely to secure the end in view. What certainty could we have that the spore—in the only useful condition for experiment—the freshly emitted state—would be in the "one or two drops?" Spores in various stages of development undoubtedly would be there. But I make no affirmation about these. There can scarcely be a doubt that germs *partly developed* must succumb at a far lower temperature. Then it follows that the certainty and the uniformity of results must depend upon the certainty we possess, as to whether the spores were in the fluid before heating or not. And when we remember that the phenomenon of fission may, in some monads, go on in a drop of fluid under examination as the sole method of increase for a week or more; and that, in all cases the production of the spore is extremely rare in relation to the other morphological changes, the force of this will be even more manifest.

But in thinking out the best method of determining as delicately as possible the thermal death-point of the adult, it occurred to me, that having demonstrated the existence of the spore, and having watched the development after it had endured given heat-conditions, that it would be possible to adopt means for comparing the differences that might exist between the destructive influence of a given temperature endured in "dry" and moist conditions respectively.

My plan was to arrange the form of the vessel in which the fluid was heated, so that without subsequent transference or exposure the fluid could be directly examined by the lens. This end was secured by means of extremely delicate glass blowing. The apparatus is presented in diagram at figs. 26 and 27.* In fig. 26 A is a hollow bulb, intended as a reservoir for an infusion containing any required organism or organisms. The infusion is put in through the funnel and tube B. The bulb A opens into a tube D on the opposite side,

on the 77th page of the paper, and will be referred to in a paper which I hope shortly to send to the Royal Microscopical Society.

* A large number of these pieces of apparatus have been made for me by a friend, an amateur. But for exquisitely finished and accurate work I have been enabled to obtain them nowhere as they are produced by Mr. Gimingham, whose remarkable dexterity in the production of the radiometer bulbs is well known. He has from time to time made them for me, in various forms, and in a manner that leaves nothing to be desired. The principle of the apparatus is one that was adopted jointly by Dr. Drysdale and myself in the study, still proceeding, of the bacteria. But the forms of the apparatus, as described above, were made for these present investigations.

and this tube terminates in a delicate closed and flattened cell C. In a microscopical point of view this cell is the most important part of the apparatus. It is a flattened bulb, and its upper and under walls are films of glass, varying in different pieces of the apparatus from the $\frac{1}{50}$th to the $\frac{1}{200}$th of an inch in thickness; and the space between these walls may vary from the $\frac{1}{20}$th to the $\frac{1}{100}$th of an inch in depth.

We thus have a perfect cell, completely closed, the contents of which can readily be studied with the aid of the most powerful lenses. The walls of the cell are of course not as absolutely even and smooth as the thin "covering glass" usually employed with high power lenses: but it is in the majority of cases beautifully level and clear, when the manner of its production is remembered. Of course I should never have employed these cells for the purpose of discovering delicate and unknown details; but they answer admirably for determining the presence or absence of phenomena, the nature of which is well known beforehand.

It is manifest then, that if a fluid be put in the bulb and stand at the level of the dotted line A, that it will fill and be in communication with the cell C.

E is a hollow bulb filled with calcined air, and hermetically sealed; but it does not communicate with the bulb A on account of the presence of a thin glass partition or septum F. The object of this is, that when by boiling, the air has been driven out of the bulb A, and the whole interior space has been hermetically sealed at J, air may be again introduced; which is accomplished thus: H is a pointed piece of platinum wire, heavy enough by a sharp shake to break the septum F, but too large in diameter to pass through the neck G, but of course the calcined air immediately enters and restores to the fluid its normal conditions.

Fig. 27 is precisely the same as the above, the same letters referring to the same parts in both; but it has an addition to it marked MN. This is a tube opening at N into the bulb A, but until needed the communication of the tube with the interior of the bulb is prevented by the thin partition L. The object of this is, that supposing a given infusion to have become sterilized in relation to a certain organism, by any ascertained temperature, to determine whether nevertheless the fluid is still capable of *sustaining* the organism if it be reintroduced. To do this, a piece of platinum wire, as before, is taken and touched with a fluid in which the living organism abounds; it is then placed in the tube K. The tube is then sealed at M; the piece of platinum is shaken sharply, breaks the septum L, and falls into the fluid, inoculating it. In relation to the particular organism now being considered, this, however, was not required.

The first important matter to be taken into account was the death point of the adult. A piece of apparatus similar to fig. 26 was used.

When the fluid containing the organism was inserted, a large number of the organisms was seen by the lens in the cell C. The whole apparatus was now placed in a copper vessel with cold water, the water just covering the bulb A, and a thermometer was placed in the water. A Bunsen's burner was used for heating, which had minute jets springing from a tube bent in the form of a parallelogram, corresponding to the form of the vessel. The heat was now applied, so that the water rose in temperature with extreme slowness. It was taken out directly the thermometer read 100° F., and at once examined. Several of the organisms were at once visible: but the great majority of them were quite still, and the only movement discoverable was a more or less languid movement of flagella, the bodies being motionless. In seven minutes a form sailed slowly across the field, but I preferred to keep my attention fixed on those which I had seen from the first. In the course of three-quarters of an hour the flagella of ten forms were all vigorously working, and the bodies began to move, and in an hour and a-half from the time of being taken out, the whole field was as active as before heating.

In the same manner the fluid was next raised to 130° F. On examination the immediate effect was more marked, the inaction more complete. Only one flagellum was seen to move in the whole field. But in the course of half an hour the flagella were gently swaying, and in two hours the same individuals were swimming freely.

When, however, the fluid was raised to 140° F., the inaction was absolute when the cell was first examined, and only one-fourth of the individuals in a selected field revived. These did so in the course of three hours, but the remainder did not move to the end of the day, and were in the same position and inactive on the morrow; and it was subsequently shown, by the same means, that a temperature of 142° F. was entirely destructive of the vitality of the adult.

The next step was to ascertain the effect of the boiling point upon the *spores*.

To be quite sure that spores, in the condition required, were in the fluid, a thin circle of glass was cut that would lie upon and take the place of the ordinary floor of the "continuous stage." By this means if a deposit of spores were seen to take place, it could be readily removed bodily from the microscope. On the under surface of this circle of glass a few lines with the diamond were made, in convenient places, to facilitate its subsequent fracture. On this then, in the stage, properly covered as usual, the fluid was hunted and watched, and fresh drops taken until a cyst was seen, and then watched until it burst. The circle of glass on which this happened was now taken carefully away from the microscope and broken, so that the pieces containing the fluid drop fell into the funnel B, fig. 26. The fluid containing the organism in abundance was now poured over these down the tube and

into the bulb A, carrying with it the fluid on the fragments of glass which contained the just emitted spore. Thus it was known that spores in the condition required were actually in the apparatus.

The whole was now placed in a suitable vessel with solid white paraffin, which was slowly melted, covering when in a fluid state the whole bulb A. The heating of the paraffin was extremely gentle, and it was raised as much above the boiling point of water as was consistent with a *gentle* ebullition of the infusion A. This was continued for ten minutes. The tube J was then hermetically closed before the ebullition had ceased, and was removed from the paraffin and carefully wiped while hot. The piece of platinum H was now shaken sharply upon the septum F, and the calcined air contained in E was admitted into the bulb A, the platinum remaining on the top of the passage G, which was too small to admit of its entrance into A. In this way the normal conditions of the infusion were as far as possible restored.

The cell C was now fixed upon the stage of the microscope, and could be carefully examined with the most suitable lenses; and from the fact that the cell was closed, "immersion" lenses could be employed, which gave an advantage in working.

I first employed an immersion $\frac{1}{16}$th. But nothing was at all visible but Brownian movement, and during the next seven hours no trace of the organism could be found. The whole was now left for six hours more, and then the entire cell was carefully "searched" with an immersion $\frac{1}{12}$th. At the close of an hour and a half three of the organisms, one in the adult condition, and two in the semi-developed state shown in fig. 24, were found swimming freely; and in the course of the next few hours the cell was freely visited by the organism in full vigour.

The length of time that elapsed before the organism appeared in the cell, I presumed to be explicable by the fact, that it took a considerable time for it to arise, in sufficient quantity, in the reservoir A to render it probable that it would migrate into the cell C; and this supposition was sustained by subsequent facts.

It was clear, then, that 212° F. was a temperature not destructive of the germ when endured in a fluid condition.

To reach higher temperatures it was, of course, needful to employ a digester. For this purpose tubes alone were employed, which were terminated in the cell C, fig. 26, but the bulb A and all its appendages were dispensed with; the tube D, for example, terminating at one end in the delicate cell C as before, but at the opposite end in the place of A was a simple funnel for the delivery of the infusion into D. When the spore in the required condition had been found and placed in the funnel as before described, the fluid was inserted as before, and the tube was then hermetically sealed.

It was now placed in a small digester, with a register for pressures,

and heated up to the temperature of 240° F., 10° F. below the death-point in the dry condition. It was kept at this for five minutes.

As soon as it could be conveniently taken from the digester it was placed in a "cradle" on the stage of the microscope. Nothing but violent Brownian movement was visible anywhere; and nothing more than this could be seen by the end of twelve hours, although the search was very strict and systematic. For the next twenty-four hours no other result ensued, and at the end of six days there was no trace of the organism. The question now arose as to whether any incapacity to sustain the organism had been superinduced. For this purpose the tube was opened at its sealed end, and a small piece of platinum wire which had been immersed in the fluid containing the living organism was dropped into it. It was then sealed again and examined. In the course of twenty minutes seven of the introduced organisms were seen, and in the next two days they were present in great abundance.

It thus became manifest that the previous sterility of the fluid in the cell was due to the destruction of the germs, or spores, by the heat to which the infusion had been subject.

Another tube was now taken, and after preparation in all respects in the same way, was heated in the digester to 230° F., and kept at that for five minutes.

The result was precisely the same as in the preceding instance. There was no trace of the living organism from the first, on to the end of a week. But in this case also, inoculation reintroduced it successfully, and it flourished.

I now determined that 220° F. should be tried; and after the tube and its contents had been arranged as before, it was heated to that point, and kept in that condition for five minutes.

When first examined with a suitable lens, as before, nothing but the well-known Brownian movement was visible. And this remained true for the first five hours, although the whole cell was carefully examined with the new formula $\frac{1}{12}$th and $\frac{1}{16}$th inch objective, and repeatedly "searched" with the $\frac{1}{8}$th. But during the sixth hour I became convinced that the organism in a developing condition was present; it was in a part of the cell immediately under the tube; and in the course of a few minutes I was perfectly satisfied that the organism in the condition represented at fig. 22 was there, but only five could be seen; these, however, increased in size as usual, and in the course of forty minutes three of them began gracefully to move, and following one of them I saw it attain to the usual adult condition. The next day the cell had scores of the organisms swimming in it.

This experiment was now repeated, every detail of preparation being as before. The results were similar; that is to say, the living organism reappeared; but it was not found until the seventh hour, and then it was freely swimming and in an almost perfect condition. But I could

not at this time find more than this one; but in the course of some hours several more were present, perhaps as the result of fission.

From this it appeared plain that the spores had survived a temperature of 220° F. in a fluid heat: but by a series of subsequent tests conducted in precisely the same way, I found that if 220° F. might not be considered the limit of temperature which the spore could survive, at least this organism never showed itself again after heating the spore to 222° F.

It thus appears that in this particular organism the dry heat is considerably less destructive of the vitality of the spores than the moist heat. That there is, indeed, a difference of 28° or 29° F.; or, speaking broadly, we might say 30° F.

This is undoubtedly a difference of considerable importance. But a temperature of 220° F. resisted successfully at all, implies of necessity actual protection of some kind. I shall not attempt to theorize upon or suggest what that may be; but it is manifestly not beyond the reach of chemical and physical science to approximate to an explanation, whilst biological science furnishes analogy in higher and more complex departments of its researches. One thing is absolutely certain; which is, that the *optical condition* of the freshly emitted spore is quite distinct from that which it presents in from three-quarters of an hour to an hour after development has commenced. It is to the comparative opacity of the newly emitted germs that their visibility at that time is due. Hence they are most certainly discovered by means of a shaft of aln ost parallel light the $\frac{1}{100}$ of an inch in diameter at right angles to the plane on which the spores are lying, and their imperfect transmission of this reveals them. But in the course of an hour they become far more transparent than even the adult form. This is undoubtedly the result of the vital processes involved in germination. The sarcode is in a fluid condition and cannot resist the heat that surrounds it—a constant current of liquid must be passing in and out of the sarcode by imbibition and exosmosis. The result must be the circulation through the sarcode of fluid at a temperature destructive of the vital processes. Hence the destruction of the adult at 142° F., and there can be little doubt but the young germinating form would be destroyed, for the same reasons, at the same or—perhaps from the probably greater intensity of the vital processes—even a lower temperature. But in the spore, the vital activities of the developing protoplasm have not commenced; the sarcode is in all probability in a fixed state; and a protective condition of that sarcode, resisting the diffusion of heat through it, is by no means difficult to understand. Indeed it will be palpably of service to the organisms, and like desiccation enable them to overcome the extreme vicissitudes of condition in temperature, drought, and so forth, to which over the surface of the earth, they must, as septic

organisms, be exposed. But from a series of experiments on the spore of such of the "monads" whose Life-Histories were worked out by Dr. Drysdale and myself, as I have been enabled to get again during the last two years, I have been able to satisfy myself that 30° F. is by no means the fixed difference between the power of thermal resistance possessed by the spores of these forms when heated in a dry and a moist condition. There is considerable variety in this matter, and variety which in all probability, nothing but a perfect acquaintance with the vicissitudes through which by adaptation they have survived in their evolutional history, could explain.

In conclusion, without entering into details, I may observe that these forms have, as far as my observations have extended, a far greater capacity to resist heat than the bacteria; but their distribution is far more limited.

DESCRIPTION OF PLATES.

Plates 8 and 9.

Figure 1. The normal form of the organism described; *d* being the nucleus.

Figure 2. The representation of the mode in which it appears by mechanical means to break up the decomposing tissues of the animal matter in the maceration. *a*, *b*, are points at which its lateral flagella are "anchored;" it then draws its body down, coiling the flagella into spirals seen at *c*; it then springs up and forward in the direction of the arrow *d*, and with the flagella as a radius darts rapidly down upon a piece of tissue in the arc of a circle shown by the arrow *e*.

Figure 3. The first indications of fission are seen at *a*, *b*, and the nucleus has moved to the centre.

Figure 4. Fission is in progress as seen at *a* and *c*, and in the nucleus *b*.

Figure 5. It is still progressing; a pale line runs through the dividing body, and the nucleus has separated.

Figure 6. Fission still further advanced.

Figure 7. Only a neck of sarcode now unites the bodies; this is sharply pulled, and results in a thin fibre connecting two forms as seen in fig. 8.

Figure 9. This has stretched as here drawn; the bodies pulling in the direction of the arrows, until the fibre snaps in the middle, and two perfect forms go free.

Figure 10. At the terminus of a series of fissions in about a third of the cases, the body becomes changed, slow of movement, and amœboid, as here drawn: and then rapidly changes into the shape seen in fig. 11.

Figure 12. The form shown at fig. 11, after forming a band seen at *a* goes into the midst of a group in the "springing" state, and speedily unites itself to one, which at once goes free; and they swim together as in this figure.

Figures 13, 14, 15, 16, 17. Represent different progressive stages of fusion, which terminate in a still sac (15), which ultimately opens and pours out spores (16, 17).

Figures 18—25. Different progressive stages in the growth of the emitted spores.

Figure 26. A piece of glass apparatus for testing the thermal death-point of the adult, and for discovering whether or not the boiling-point in a fluid destroys the germ. The cell *c* enables this to be determined by micro-

scopical examination directly, without any transference of the infusion; and the bulb E being filled with calcined air, enables the experimenter, when the whole piece of apparatus is closed at J, to restore air to the bulb A containing the infusion, by breaking the thin septum F by means of the comparatively heavy piece of platinum H, which opens a communication between E and A.

Figure 27. Is the same as the above, except for the added tube MN. This is intended to be used if the fluid should prove to be sterilized, for the purpose of inoculating it again by means of a piece of platinum which is charged with the fluid in which the organism is abounding. This is introduced into the tube, which is at once closed at M. It is then made to break the septum L, and falls into the fluid inoculating it, to discover if it still has the power to sustain the organism.

All the drawings of the organism are magnified 3,000 diameters, except where otherwise indicated on the plates.

II. "On the Reversal of the Lines of Metallic Vapours." By G. D. Liveing, M.A., Professor of Chemistry, and J. Dewar, M.A., F.R.S., Jacksonian Professor, University of Cambridge. No. II. Received March 26, 1878.

Since our last communication to the Society we have succeeded in reversing characteristic lines of the vapours of rubidium and cæsium. Considering the known volatility of these elements, and the small quantity of their compounds at our disposal, we thought it better to try the effects first in glass tubes. For this purpose a piece of combustion tubing had one end drawn out and the end turned up sharply, and sealed off (like an ill-made combustion tube of the usual form) so as to produce an approximately plane face at the end of the tube; a small bulb was then blown at about an inch from the end, and the tube drawn out at about an inch from the bulb on the other side, so as to form a long narrower tube. Some dry rubidium or cæsium chloride was next introduced into the bulb, and a fragment of fresh cut sodium, and the narrow part of the tube turned up, so as to allow the tube and bulb to be seen through in the direction of the axis of the tube. The open end was then attached to a Sprengel pump, and the air exhausted; the sodium was then melted, and afterwards either dry hydrogen or dry nitrogen admitted, and the end of the tube sealed off at nearly the atmospheric pressure. We found it necessary to have this pressure of gas inside the tube, otherwise the metal distilled so fast on heating it that the ends were speedily obscured by condensed drops of metal. Through these tubes placed lengthways in front of a spectroscope, a lime light was viewed. On warming the bulb of a tube in which rubidium chloride had been sealed up with sodium, the D lines were of course very soon seen, and very soon there appeared two dark lines near the extremity of the violet light, which, on measure-